Divisional morphogenesis of *Notohymena australis* (Foissner & O'Donoghue, 1990) Berger, 1999 (Ciliophora, Hypotrichida, Oxytrichidae)*

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Abstract: The morphology and the divisional morphogenesis of the freshwater hypotrich *Notohymena australis* have been investigated using life observation and protargol impregnation. The morphogenesis commences with a de novo proliferation of basal bodies for the opisthe's oral primordium at three spots on the ventral surface between postoral ventral cirrus V/3 and the uppermost pretransverse ventral cirrus V/2. The six fronto-ventral-transverse-cirri anlagen develop in the usual oxytrichid mode, except the proter's and opisthe's anlagen V and VI which show an unusual development. In early stages they are distinctly connected and later separated, when differentiation of cirri commences. Marginal rows and dorsal kineties develop within the parental structures, dorsomarginal rows develop from the right marginal row. At the end of dorsal kineties 1, 2, and 4 more than one caudal cirrus is produced. During morphogenesis the proter's oral structures show some signs of cryptic reorganisation, indicated by the flattening of the buccal cavity and the parallel arrangement of the reorganised paroral and endoral membrane.

Key words: Cell division, ontogenesis, primary primordia, Stichotrichia, stomatogenesis, taxonomy.

Introduction

The shape and arrangement of the paroral and endoral membrane are useful characters for discriminating oxytrichid hypotrichs (Diesing 1866; Kahl 1932; Blatterer & Foissner 1988; Foissner 1989; Berger & Foissner 1997; Berger 1999). Blatterer & Foissner (1988) established *Notohymena* for species having an almost hemispherically curved paroral membrane, whose distal end is broadened and ventrally hooked. This important character is recognisable only after protargol impregnation.

Up to this day, five species have been placed in *Notohymena*: *N. selvatica* (Hemberger, 1985) Blatterer & Foissner, 1988; *N. rubescens* Blatterer & Foissner, 1988 (type species); *N. australis* (Foissner & O'donoghue, 1990) Berger, 1999; *N. antarctica* Foissner, 1996; and *N. pampasica* Küppers, Claps & Lopretto, 2007. The world wide second record of *N. australis* was provided by Foissner & Gschwind (1998); a detailed morphogenetic description of this species is lacking. Therefore, the present paper provides descriptions of the non-dividing cells and of the morphogenesis of *N. australis*.

Material and methods

Notohymena australis was found in August 2006 in the rotted material taken from the ground of my freshwater aquarium, probably imported by tropical plants and fish. Several specimens were isolated and cloned; three clones could be established successfully in all. Cultures were set up in Petri dishes containing non-carbonated mineral water ("Vittel") and one rice grain to support growth of indigenous bacteria and small ciliates which served as food organisms. Additionally, every second to third day three to four drops of *Chlorogonium elongatum* concentrated by centrifugation were added.

Body shapes of living specimens were drawn from slides without cover glasses. Details were studied on slightly to heavily squeezed individuals using an oil immersion objective. The morphogenetic events during binary fission were investigated with well fed cells, usually prepared one day after feeding with *C. elongatum*. Staining was performed according to the protargol protocol of Wilbert (1975). Drawings were made with the help of a camera lucida.

To make plain the changes during morphogenetic processes, old cirri are depicted by contour, whereas new cirri are shaded black. Statistical procedures and terminology are according to Sokal & Rohlf (1981) and Berger (1999).

* The author would like to dedicate this paper to Prof. Wilhelm Foissner on the occasion of his 60th birthday in recognition of his contribution to protozoology, protistan taxonomy and teaching.

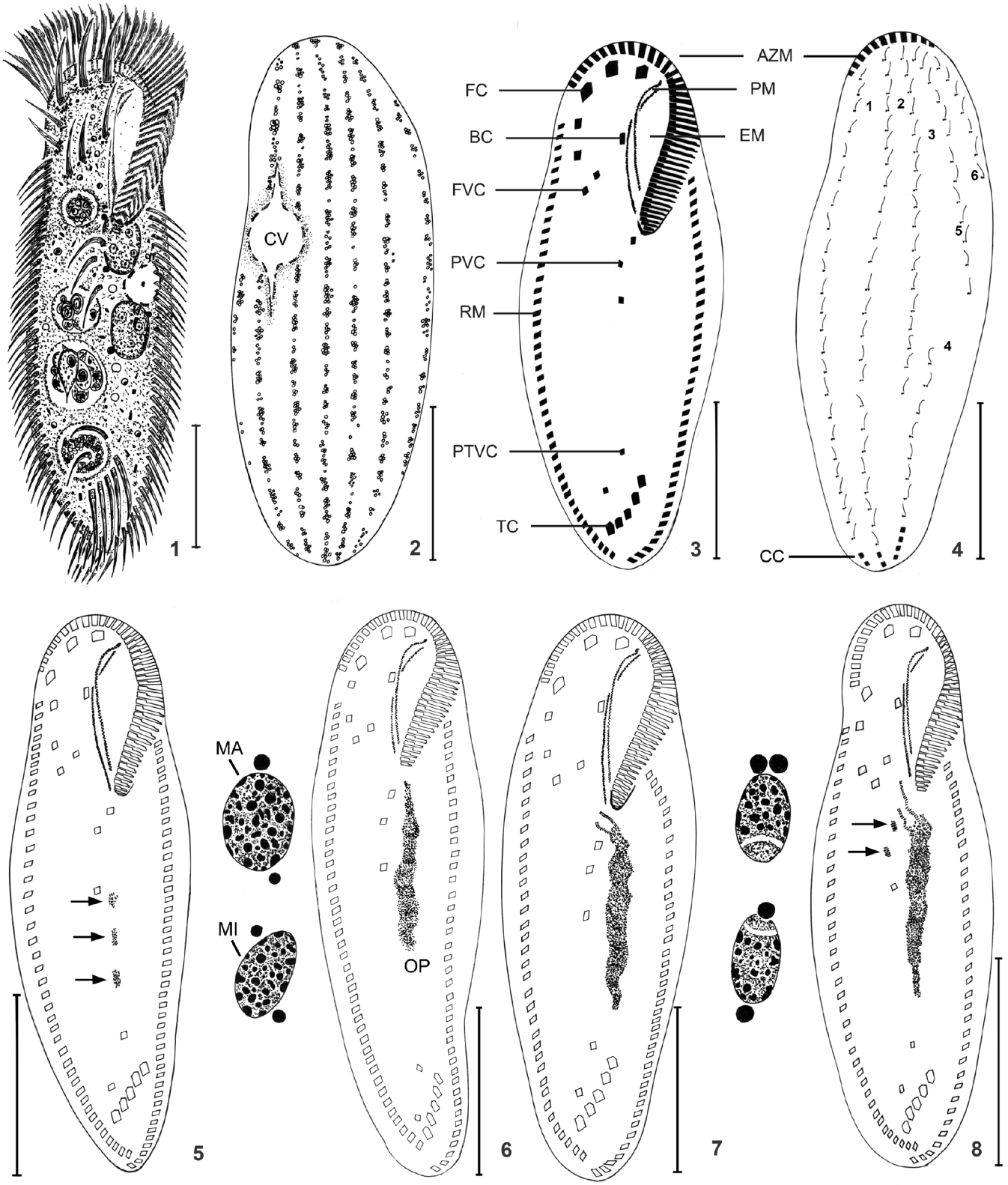

Fig. 1–8: Interphase morphology (1–4) and morphogenesis (5–8) of *Notohymena australis* from life (**1, 2**) and after protargol impregnation (3–8). **1**: Ventral view of a representative specimen. **2**: Dorsal view showing the arrangement of cortical granules and contractile vacuole. **3, 4**: Infraciliature of ventral and dorsal side of same specimen. **5–8**: Early dividers. Arrows in (5) mark basal bodies generating the oral primordium. Arrows in (8) indicate dedifferentiation of postoral ventral cirri IV/2 and V/4. AZM – adoral zone of membranelles, BC – buccal cirrus, CC – caudal cirri, CV – contractile vacuole, EM – endoral membrane, FC – frontal cirri, FVC – frontoventral cirri, LM – left marginal row, MA – macronuclear nodule, MI – micronucleus, OP – oral primordium, PM – paroral membrane, PVC – postoral ventral cirri, PTVC – pretransverse ventral cirri, RM – right marginal row, TC – transverse cirri, 1–4 – dorsal kineties, 5, 6 – dorsomarginal kineties. Scale bars: 30 µm.

Results

Interphase morphology of the German aquarium population of *Notohymena australis* (Fig. 1–4; Tab. 1)

Body size in vivo about 140–160 × 40–50 µm. Body flattened about 2:1 dorso-ventrally, distinctly flexible. Cortical granules yellow-green. Two macronuclear nodules, four to eight micronuclei. Contractile vacuole located slightly ahead of mid-body near left cell margin.

Adoral zone of membranelles occupies about 35 % of body length in vivo and 30 % on average in protargol preparations; composed of 38–45 membranelles. Buccal field moderately wide and deep and partially covered by a hyaline lip; its upper portion distinctly curved to the left. All cirri arranged in usual oxytrichid pattern; each two to four caudal cirri at end of dorsal kineties 1, 2 and 4.

Divisional morphogenesis of *Notohymena australis* (Fig. 5–23)

Division of nuclear apparatus

The nuclear apparatus divides in the usual way and hence requires no further comment (Fig. 5, 7, 9, 13–15, 18, 23).

Stomatogenesis

The first morphogenetic event is the occurrence of three groups of scattered pairs of basal bodies on the ventral surface between the postoral ventral cirrus V/3 and the uppermost pretransverse ventral cirrus V/2 (Fig. 5). The basal bodies increase in number and form an anarchic field (oral primordium). The oral primordium is anteriorly broadened and posteriorly narrowed (Fig. 8).

Further proliferation of basal bodies and membranellar differentiation produce the new adoral zone of membranelles for the opisthe. Soon, the differentiation of membranelles proceeds posteriadly, while the primordium for the two undulating membranes separates in form of loosely arranged pairs of basal bodies at the right side of the differentiating adoral zone of membranelles (Fig. 12–15). The parental adoral zone of membranelles is retained. The anterior portions of the parental undulating membranes reorganise more or less simultaneously, thereby lying side by side and showing some proliferation of basal bodies at their anterior ends. Later the first frontal cirrus I/1 for the proter is generated from this arrangement (Fig. 12–15).

Development of the cirral primordia

In the proter, the buccal cirrus II/2 and the frontoventral cirri III/2 and IV/3 develop into anlagen II, III and IV. Anlage I develops from the reorganising undulating membranes (Fig. 9–11).

The oral primordium generates the cirral anlagen II and III at the anterior end (Fig. 7), whereas the postoral ventral cirri IV/2 and V/4 are transformed into cirral primordia for the anlagen IV and V (Fig. 8). At last, the postoral ventral cirrus V/3 is changed into a primordium, which generates the cirral anlage VI (Fig. 9, 10). Cirral anlage I is separated from the developing undulating membranes (Fig. 11, 12, 17, 18).

The opisthe's anlagen V and VI elongate and extend to the right on a level of the proter's anlagen III and IV (Fig. 10–13). Later, these elongated and posteriorly connected primary primordia are separated into a set of the two anlagen V and VI each for proter and opisthe (Fig. 14, 15).

Development of the somatic primordia

The anlagen for the new marginal cirri are formed within the parental marginal rows. The proter's primordia originate from the first and/or second cirrus of the left respectively from the first (Fig. 13) or third (Fig. 15) cirrus of the right marginal row (Fig. 13, 14). The opisthe's primordia for the marginal rows are produced near the prospective division furrow (Fig. 13–15).

The anlagen for the new dorsal kineties originate within the parental dorsal kineties 1, 2 and 3 (Fig. 16, 19). The new dorsal kinety 4 is separated from the developing kinety 3 (Fig. 22). In addition, two short kineties arise from primordia which are generated de novo close to the anterior end of the new right marginal rows (Fig. 18, 20, 21). These dorsomarginal kineties move from the ventral to the dorsal surface and form the posteriorly shortened dorsal kineties 5 and 6 (Fig. 22).

New caudal cirri are produced by proliferation of basal bodies at the proximal ends of the developing dorsal kineties 1, 2, and 4. Two to four cirri caudal cirri are produced in each anlage (Fig. 22).

Discussion

Interphase morphology

The German aquarium population of *Notohymena australis* matches the German population studied by FOISSNER & GSCHWIND (1998) as well as the type population from Australia (FOISSNER & O'DONOGHUE 1990). The figures and the short description of the interphase morphology should thus suffice to orientate the reader. It is worth noting that the body shape of specimens of the German aquarium population is more slender than that of the German population and the Australian population. *N. australis* can be distinguished from the recently recorded *N. pampasica* KÜPPERS,

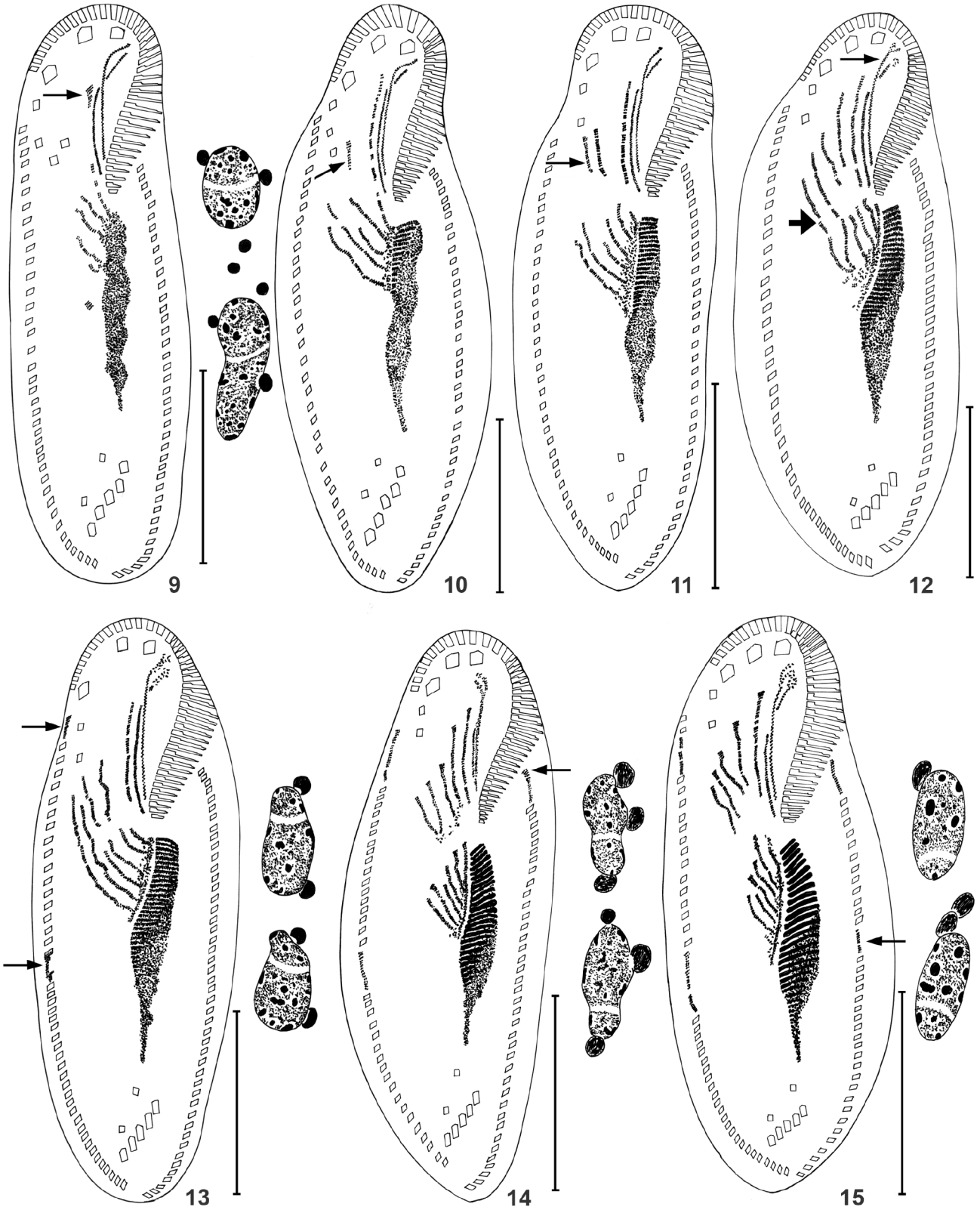

Fig. 9–15: Early (9–11) and middle (12–15) dividers of *Notohymena australis* after protargol impregnation. **9**: Arrow marks parental buccal cirrus II/2 disaggregating into a primordium. **10, 11**: Arrows indicate frontoventral cirrus III/2 (10) and cirrus IV/3 (11) becoming a primordium. Note that opisthe's cirral anlagen V and VI are long compared to anlagen II to IV. **12**: Middle divider showing reorganizing undulating membranes of the proter (small arrow). Note that proter's and opisthe's anlagen V and VI are almost connected (large arrow). **13–15**: Middle dividers showing the development of the complete set of six cirral anlagen each in proter and opisthe. Note that the connected anlagen V and VI now are separated as shown in (14). Arrows indicate disaggregation of some cirri of the right (13) and left (14, 15) marginal row. Scale bars: 30 µm.

Table 1: Morphometric characteristics of *Notohymena australis*.[1]

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length[2]	169.2	175.0	22.5	4.5	13.3	125.0	215.0	25
Body, length	167.1	160.0	31.5	6.3	18.8	112.5	207.5	25
Body, width[2]	38.8	40.0	5.6	1.1	14.5	35.0	50.0	25
Body, width	42.2	40.0	8.7	1.7	20.7	30.0	52.5	25
Macronuclear nodules, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	25
Macronuclear nodules, length	23.3	25.0	5.6	1.1	24.1	20.0	30.0	25
Macronuclear nodules, width	11.8	12.5	2.2	0.4	18.9	10.0	17.5	25
Distance between macronuclear nodules	20.4	19.2	7.6	1.5	37.3	8.0	40.0	25
Micronuclei, number	5.6	6.0	1.8	0.4	32.6	3.0	8.0	25
Micronucleus, diameter	2.5	2.4	0.8	0.2	31.9	1.6	4.0	25
Adoral membranelles, number	42.1	42.0	2.2	0.4	5.2	38.0	45.0	25
Adoral zone of membranelles, length	49.6	49.6	8.3	1.6	16.7	33.6	64.0	25
Right marginal row, number of cirri	39.8	42.0	8.4	1.7	21.0	33.0	49.0	25
Left marginal row, number of cirri	40.1	40.0	2.4	0.5	6.0	35.0	44.0	25
Frontal cirri, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	25
Frontoventral cirri, number	4.0	4.0	0.2	0.4	5.0	4.0	5.0	25
Buccal cirrus, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	25
Postoral ventral cirri, number	3.1	3.0	0.4	0.0	13.0	3.0	5.0	25
Pretransverse ventral cirri, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	25
Transverse cirri, number	5.0	5.0	0.2	0.4	4.0	5.0	6.0	25
Caudal cirri, number in kinety 1	2.2	2.0	0.6	0.1	26.7	1.0	3.0	25
Caudal cirri, number in kinety 2	2.5	2.0	0.5	0.1	20.2	2.0	3.0	25
Caudal cirri, number in kinety 4	3.6	4.0	0.6	0.1	16.3	2.0	4.0	25

[1] Data are based on randomly selected, protargol-impregnated, and mounted specimens from exponentially growing cultures. Measurements in µm. CV – coefficient of variation in %; M – median; Max – maximum value; Min – minimum value; n – number of individuals investigated; SD – standard deviation; SE – standard error of arithmetic mean; $\bar{x}$ – arithmetic mean.
[2] In vivo measurements.

Claps & Lopretto, 2007 by the characters colour of cortical granules (yellow-green vs. colourless), number of micronuclei (three to eight vs. two, rarely three) and number of caudal cirri (up to ten vs. three).

Oral primordium and oral structures

Stomatogenesis in *N. australis* commences de novo on a level between postoral ventral cirrus V/3 and the uppermost pretransverse ventral cirrus V/2, which is not in accordance with the observations in the type species *N. rubescens* where the oral primordium is generated close to the uppermost transverse cirrus II/1 (Voss 1991b). The mode of oral primordium formation occurring in *N. australis* has been observed in *N. selvatica* (Hemberger 1982), in the related *Cyrtohymena muscorum* (Voss 1991a), and in many other oxytrichids, for example, *Oxytricha*, *Gonostomum*, *Urosoma* and *Stylonychia* (Hemberger 1982; Foissner 1983; Foissner & Adam 1983a, b; Wirnsberger et al. 1985, 1986; Ganner et al. 1986; Berger & Foissner 1997; Berger 1999). Both types of oral primordium formation even occur in species of the same genus, indicating either misclassification of species or rather a random distribution of this character within the oxytrichids.

The reorganisation of the undulating membranes in *N. australis* corresponds to the processes in *N. rubescens* and of other members of the family, e. g. *Cyrtohymena* and *Steinia* (for review see Berger 1999). The genus specific pattern of the undulating membranes is obtained in *N. australis* shortly after cell division. These results are also in accordance with the observations in *N. rubescens*, *Cyrtohymena muscorum* and *Steinia sphagnicola*, suggesting that this character is a young evolutionary acquisition (Voss 1991a, b, Voss & Foissner 1996).

Development of cirral primordia

The observations on *Notohymena australis* show, that the origin and development of the six cirral anlagen occur as described in many other oxytrichids. The proter's anlagen I to IV are generated from the reorganising undulating membranes, from the buccal cirrus II/2 and from frontoventral cirri III/2 and IV/3. The opisthe's anlagen I to VI are generated from the developing undulating membranes (I) respectively from the developing oral primordium (II and III) and from the three postoral ventral cirri (IV to VI). The anlagen V and VI of *N. australis* develop like primary primordia, which is reminiscent of *Gonostomum*, *Urosoma*, and *Tachysoma* (for

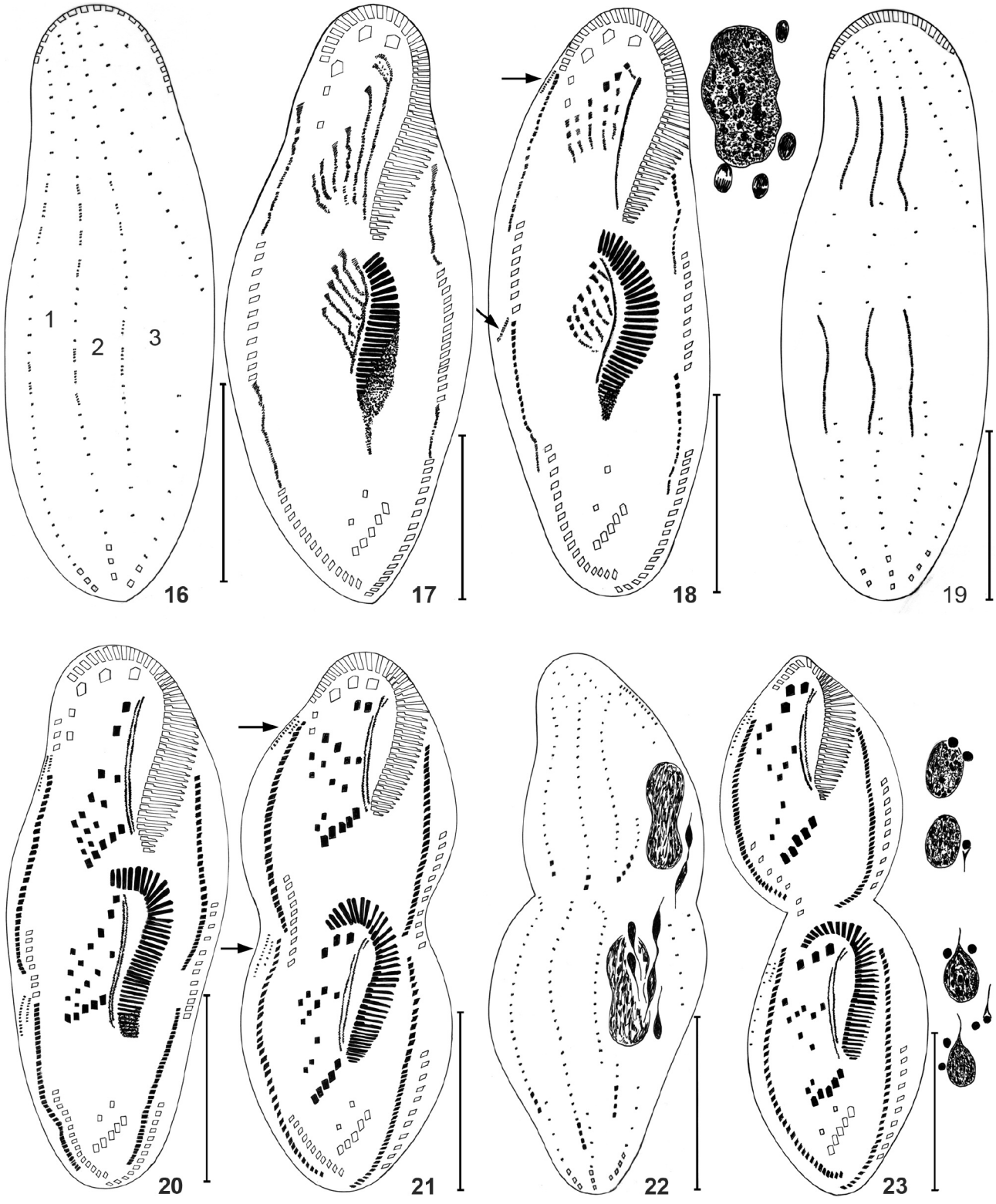

Fig. 16–23: Middle (16–19) and late dividers (20–23) of *Notohymena australis* after protargol impregnation. **16, 19**: Dorsal view showing the development of primordia within dorsal kineties 1 to 3. **17, 18**: Middle dividers showing development and segregation of cirri in the complete anlagen sets of proter and opisthe. Note that cirral anlage I is generated from reorganized undulating membranes of the proter respectively from the anlage for the undulating membranes of the opisthe. Arrows indicate the development of primordia for dorsomarginal kineties. **20, 21**: Late dividers showing further segregation of cirri and the developing dorsomarginal rows 5 and 6 at the right side of the cell (arrows in 21). **22**: Dorsal view showing more than one caudal cirrus each in new dorsal kineties 1, 2, and 4 which splits off from kinety 3. **23**: Very late divider. Most parental cirri have been resorbed. The curved paroral membrane crosses (optically intersects) the endoral membrane in the anteriormost portion; the genus specific arrangement of the undulating membranes is not finished yet. 1–3 – parental dorsal kineties. Scale bars: 30 µm.

review see BERGER 1999). But this type of anlagen formation has never been observed in *Notohymena*, *Cyrtohymena*, and *Steinia* (VOSS 1991 a, b; VOSS & FOISSNER 1996).

Development of somatic primordia

The marginal primordia of the proter develop from the first and second cirrus of the left and from the first or third cirrus of the right marginal row in *Notohymena australis* (Fig. 13–15). In *N. rubescens*, type of *Notohymena*, the first cirrus of the left and the right marginal row develop the marginal primordia (VOSS 1991b) showing that this feature is not useful for species discrimination within the genus *Notohymena*.

The anlage for the short dorsal kinety 6 is situated near the anlage for kinety 5, but shows distinct contact neither with it nor with the marginal primordium. This is in agreement with WIRNSBERGER (1987) that this kinety is a reduced and transformed marginal row which originates de novo.

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